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(71) Applicant *(for all designated States except US)*: NORTH-EASTERN UNIVERSITY [US/US]; 360 Huntington Avenue, Boston, MA 02115 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: KARGER, Barry, L. [US/US]; 62 Deborah Road, Newton, MA 02159 (US). FELTEN, Chantal [LU/US]; 140 West Canton Street, Boston, MA 02118 (US). FORET, Frantisek [CZ/US]; 525 Highland Avenue, #40, Malden, MA 02148 (US). ZHANG, Bailin [CN/US]; Apartment #1, 104 Hemenway Street, Boston, MA 02115 (US).

(74) Agents: HEINE, Holliday, C. et al.; Weingarten, Schurgin, Gagnebin & Hayes LLP, Ten Post Office Square, Boston, MA 02109 (US).

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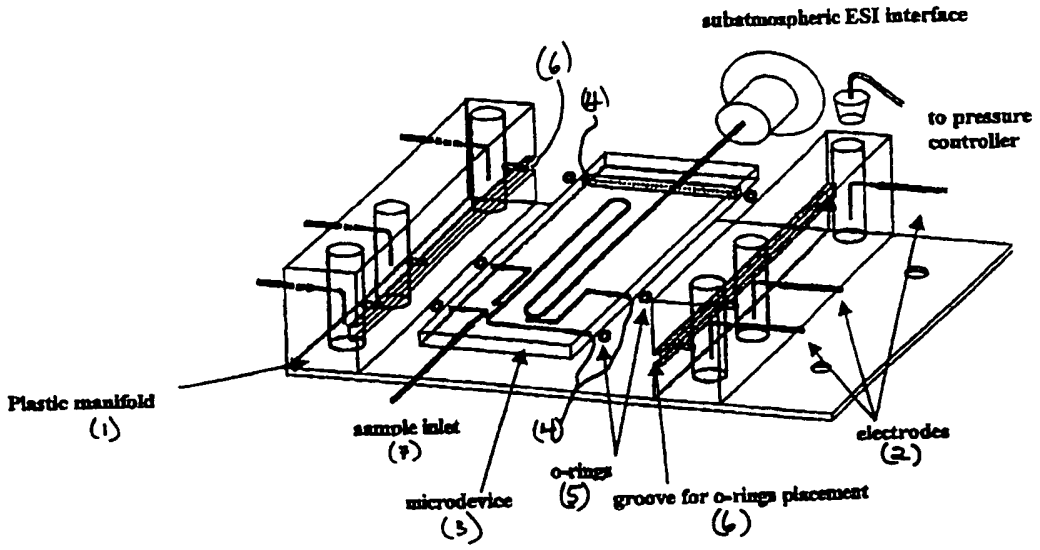

(57) Abstract

Disclosed is a liquid handling reservoir system comprising a microfluidic device (3), the device comprising multiple microfabricated channels (4), each channel having an inlet end (7); a detachable reservoir manifold (1) external to the device, the reservoir (1) having at least one opening for fluid flow into and out of the reservoir (1), the reservoir (1) being positioned so that the reservoir opening is proximate to an inlet end (7) of a microfabricated channel (4) in the device for fluid flow between the reservoir (1) and the channel (4).

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TITLE OF THE INVENTION

SYSTEM AND METHOD FOR HIGH THROUGHPUT MASS SPECTROMETRIC ANALYSIS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the priority of U.S. Provisional Patent Application No. 60/128,509 filed, April 9, 1999 entitled SYSTEM AND METHOD FOR HIGH THROUGHPUT MASS SPECTROMETRIC ANALYSIS, the whole of which is hereby incorporated by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

BACKGROUND OF THE INVENTION

Recent developments in microfabrication techniques have permitted the integration of microminiature tools for biochemical analysis within a tiny device. Complete chemical processing systems, e.g., reaction chambers, separation capillaries and their associated electrode reservoirs, as well as certain types of detectors, can be consolidated on a microchip of, e.g., a glass or fused silica. Such "labs-on-a-chip," in principle, permit effective utilization and manipulation of minute quantities of material. Systems have been developed that permit the efficient transfer of nanoliter quantities or other small quantities of a fluid sample from the spatially concentrated environment of a microscale device

to "off-chip" analytical or collection devices without an increase in sample volume. (See, U.S. Patent No. 5,872,010, the whole of which is hereby incorporated by reference herein.) However, methods for moving small quantities of samples from a sample vial or holder onto a chip or directly to an analytical device without either evaporation or dilution are less well developed.

SUMMARY OF THE INVENTION

In one aspect the invention is directed to a liquid handling infusion system comprising a microscale sample holder configured for holding multiple samples, the holder being situated in conjunction with a positioning system; a sample infusion capillary having an inlet end and an outlet end, the inlet end of said capillary being in alignment with one of the multiple samples in the sample holder; and a source of positive or negative pressure for applying the positive or negative pressure across the sample infusion capillary. In other embodiments, the infusion system further comprises an ESI chamber coupled to the outlet end of the sample infusion capillary, the sample infusion capillary further comprises a separation capillary, or the sample infusion capillary is a channel (open or closed) in a microfluidic device.

In another aspect the invention is directed to a liquid handling reservoir system comprising a microfluidic device, the device comprising multiple microfabricated channels, each channel having an inlet end; a detachable reservoir manifold external to the device, the reservoir having at least one opening for

fluid flow into and out of the reservoir, the reservoir being positioned so that the reservoir opening is proximate to an inlet end of a microfabricated channel in the device for fluid flow between the reservoir and the channel.

The systems of the invention, alone or in combination, are suitable for moving small quantities of samples from a sample vial or holder onto a chip or directly to an analytical device, without either evaporation or dilution, and for providing for rapid and automated washing of the infusion capillaries and/or the complete system.

The samples may be transfered for electrospray-mass spectrometry analysis (ESI/MS), for atmospheric pressure-chemical ionization mass spectrometry analysis (APCI/MS), for matrix assisted laser desorption ionization mass spectrometry (MALDI/MS), for nuclear magnetic resonance analysis (NMR), for pneumatically or ultrasonically assisted spray sample handling, for transfer to an off-chip detection system, such as electrochemistry, conductivity or laser induced fluorescence, or for collection of specific fractions, e.g., in collection capillaries or on collection membranes.

The channels of any microdevice used may be arrayed in any format that allows for sequential or simultaneous processing of liquid samples.

In any embodiment, each channel may include electrical contacts, so that an electric circuit path can be established along the channel. For example, one electrical contact can be on the entrance side of a channel and another electrical contact can be on the exit

side. In an alternative arrangement, an electric circuit can be completed by an external contact, beyond the exit end of the channel. For example, if the exit port of a channel is used as an electrospray source for a mass spectrometer, the mass spectrometer sampling orifice can serve as the counter electrode. Samples can be transferred off chip for subsequent analysis by switching the electric current sequentially to each channel on the chip.

Samples can be introduced into a channel on the microscale device of the invention by a variety of methods, e.g., by pressure, electrokinetic injection, or other technique, and an electrical current and/or pressure drop can then be applied to cause the sample components to migrate along the channel. The channels may function only for fluid transfer, e.g., to a mass spectrometer, or the channels can serve as environments for various types of sample manipulations, e.g., for micropreparative or analytical operations, such as capillary electrophoresis (CE), chromatography or the polymerase chain reaction (PCR), or for carrying out any type of sample chemistry. The channels may be filled with membrane or packing material to effectuate preconcentration or enrichment of samples or for other treatment steps, such as desalting. Furthermore, other modification of sample components, e.g., by enzymes that are covalently bound to the walls of a channel or are free in a channel, are possible. Packing material may be bound to the walls of the channels or may include other components, such as magnetic particles, so that when a magnetic field is applied, the magnetic particles retain

the packing material in place. The magnetic particles can also be used for efficient mixing of fluids inside the channels, using an external magnetic field. A micromachined filter or other stationary structure may also be employed to hold packing material in place. Alternatively, stationary structures can be micromachined, cast or otherwise formed in the surface of a channel to provide a high surface area which can substitute for packing material.

A sample can be introduced into a channel in a short starting zone or can fill the whole channel completely. Filling only a small part of the channel with the sample is preferable when an on-chip separation of sample components is to be carried out, such as electrophoresis or chromatography. Filling the whole channel with the sample may be advantageous in cases when off-chip analysis requires extended sample outflow, such as sample infusion/electrospray ionization for structure analysis by mass spectrometry.

In many cases a liquid flow may be required to transport the analytes in a sample into a specific channel, or along the length of the channel, or out of the channel via an exit port. Therefore, to assist in the required fluid transfer, a pumping device may be incorporated into or associated with the microscale device of the invention. For example, a heating element can be used to cause thermal expansion, which will effectuate sample liquid movement, or a heating element can be used to generate a micro bubble, the expansion of which causes the sample to travel in the channel. Other options may include pumping by the pressure of a gas or

gases generated by on-chip electrolysis. Flow can be also generated by application of a pressure drop along a channel or by electroosmosis inside a channel.

As samples move to the end of a channel, they can be subjected to detection or analysis at a site external to the microscale device of the invention by a variety of techniques, including mass spectroscopy, nuclear magnetic resonance, laser induced fluorescence, ultraviolet detection, electrochemical detection, or the like. The end of each channel may include a tip configured to facilitate transfer of the sample volume.

The invention may be used in a fluid sheath (e.g., liquid or gas) or sheathless mode depending on the type of analysis required and the size of the sample exiting a channel. In a sheathless arrangement, the exit port is formed at the end of the channel. When a liquid sheath is required (e.g., for the addition of a liquid, a chemical and/or a standard prior to electrospray or to provide electric connection via the sheath fluid), an exit port can be created at the merge point of two channels, one supplying the sample and the other the sheath liquid. Selective analysis of analytes in both the cationic and anionic modes can be performed easily by rapid switching of the polarity of the electric field.

Different sized channels may be employed on the same microscale device. For example, larger channels may be used for cleanup operations, and smaller channels may be used for processing operations. Moreover, other operations can be performed in other regions of the device, such as chemical processing, separation, isolation or detection of a sample or a component of the

sample, prior to sample loading in a channel. Thus, it is possible to carry out sample chemistries or to conduct micropreparative and analytical operations on both a starting sample and its separated components within the device of the invention, prior to transfer of the sample or its components off chip for further analysis or collection. Additionally, detection of a sample may be carried out on the microdevice itself, e.g., by a fiber optic detection system, which can provide complementary control information for off-chip analysis and detection, or by any other suitable detector such as laser induced fluorescence, conductivity and/or electrochemical detector.

Buffer reservoirs, reaction chambers, sample reservoirs, and detection cells may also be fabricated along with each individual channel. More complex structures can be created by stacking or otherwise assembling two or more microfabricated devices. In addition, individual instrument blocks such as sample reservoirs, pretreatment or separation channels, and exit ports can be micromachined separately and combined into one complete system in much the same way as hybrid integrated circuits in electronics are formed. Microfabrication techniques are precise and will allow for a high degree of reproducibility of selected channel and exit port shapes and dimensions.

The microscale fluid handling system of the invention permits more efficient use of powerful analytical devices, such as the mass spectrometer, than is currently possible. In addition, the system of the invention can be manufactured as a disposable device that

is suitable for cost effective automation of the analysis of a large number of samples. Using this micromachined approach, high throughput analysis by mass spectrometry would be possible. In addition, handling of small volumes and quantities of samples would be facilitated, and consumption of valuable samples and reagents would be reduced. Applications include any laboratory analysis methods, especially where high throughput and minimization of cross-contamination are desirable, such as screening and diagnostic methods, and such other analytic methods as pharmacokinetics where fresh columns are required for each run.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a high throughput continuous flow electrospray interface system for mass spectrometry, according to the invention;

Fig. 2 shows the alignment of a microtiter well plate sample holder in the system of the invention;

Fig. 3 shows a flow through washing device according to the invention;

Fig. 4 shows a liquid handling reservoir system according to the invention;

Fig. 5 shows a detail of the sustem of Fig. 4 in which a detachable manifold is separated from a channel on the associated microdevice by a porous membrane; and

Fig. 6 shows the results of experiments carried out using the sustem of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

The current acceleration of pharmaceutical research directed to identifying new drug candidates and the related increase in the number of pharmacokinetic studies require suitable techniques for high throughput analysis. In addition to a number of assays based on optical measurement, mass spectrometry is gaining in importance for pharmacokinetic studies as this technique can provide sensitivity combined with speed, mass selectivity and structural information. High throughput electrospray/MS systems predominantly utilize the flow injection mode of operation. In this injection method, a zone of sample is introduced into a stream of a carrying fluid (fluid sheath) for transport into the electrospray source for ionization and analysis. Typically, a modified liquid chromatography fluidic system is used for the task. Since a flow injection system consists of a number of relatively long connection tubes, relatively large sample volumes are required for analysis. Frequently, more than 10 µL of sample is consumed per analysis, although only a fraction of this amount is required to be electrosprayed to generate useful information. One analysis cycle consists of system loop filing, injection/analysis and washing. After the analysis, the whole fluidic system must be washed to prevent contamination of the next

injected sample. Typically, analysis cycles of 40-60 sec can be achieved, but the time for actual analysis and data collection will only be a few seconds, as most of the cycle time will be used for system flushing. Thus, the main disadvantage of the flow injection system are large sample volume consumed; low duty cycle as a large part of the analysis time is lost during the system washing; time for analysis limited by sample loop size; and limited use of low volume microtiter well plates.

The infusion system of the invention, schematically shown in Fig. 1, is based on the subatmospheric electrospray interface described in pending U.S. Application No. 08/784,400, the whole of which is hereby incorporated by reference herein. The system of the invention is useful, e.g., for high throughput ESI/MS analysis of small (submicroliter) sample volumes without the abov-mentioned limitations. Referring to Fig. 1, the system of the invention consists of a microtiter well plate 2, vertically arranged on an XYZ stage 1 and interfaced to an ion trap mass spectrometer by a fixed subatmospheric ESI-microsprayer 4. The infusion flow is achieved through the application of a vacuum inside the spray chamber. A simple flow through wash reservoir 3 is inserted between the ESI-interface and the microtiter plate, allowing for external as well as internal washing of the infusion capillary. Wash reservoir 3, machined from a Teflon block, is connected by Teflon tubing to a syringe pump containing the appropriate wash solution. The infusion capillary passes through the opening of the wash device reservoir, thus allowing back and forth

movement of the wash device over the inlet of the infusion capillary.

The system uses one capillary (e.g, 10 mm - 10 cm, with an id of 10-100 μm) for both the sample aspiration and electrospray ionization. Alternatively, the electrospray chamber can be operated at atmospheric pressure, and the sample flow generated by pressurizing the sample inlet side. As shown in Fig. 2, by positioning the microtiter well plate vertically, the length of the sampling/ESI capillary can be significantly reduced, resulting in lower sample consumption. Once this capillary is dipped into the sample, the lower pressure in the ESI chamber will cause sample flow. The ESI capillary is connected to a high voltage power supply that results in electrospray of the sample at the tip. The capillary remains in the sample vial and continuously feeds sample tot he mass spectrometer until sufficient information is obtained, and the stage then moves to the next sample position for the next analysis. To avoid contamination of the next sample, the ESI capillary is washed in a flow through reservoir during the sample position change as shown in Figure 3. A computer-controlled valve can be used to adjust the pressure in the ESI chamber to control the sample and washing fluid flows, respectively.

Since the internal volume of the sample/ESI capillary is only a few nL (10-200), very small sample amounts are consumed. At the same time, the useful signal can be observed as long as required, since the sample is not carried into the electrospray as a zone in the mobile phase, but delivered as a continuous flow

directly from the sample vial. Very high duty cycle (over 80%) can be obtained and sample throughput of more than one sample per 5 seconds can easily be achieved. Since the useful MS signal can be observed as long as the ESI capillary is in the sample well, a simple control software can be used to decide if MS/MS analysis is required. There is no need for a new sample injection or flow programming which would be required in a conventional flow injection system.

As a logical extension of the technique, one can also envision that the electrospray capillary be substituted by a miniaturized separation system. For example, a separation column or a microdevice (lab-on-a-chip) suitable for sample preparation and separation (CE, CEC, LC, IEF) can be used in place of the sampling capillary for multi-dimensional analysis of complex mixtures.

The practical use of microfabricated-microfluidic devices requires reservoirs for storage of chemical reagents, electrolytes and samples to be analyzed. The devices currently under development typically integrate such reservoirs directly on the microfabricated device (typically made of glass or plastic), mostly in the form of holes drilled into the surface of the microdevice. Such a design is suitable only for disposable microdevices since such reservoirs have limited volume and cannot be easily replenished after the analysis. At the same time, the fabrication procedures (drilling and gluing) increase the cost of the microdevice.

For devices intended for repetitive analyses or handling of different samples, the volume of the on-chip

reservoirs would likely not be sufficient. Additionally, the small volume of the on-chip reservoirs may suffer from adverse effects of evaporation and electrolysis when electrokinetic transport is used. Thus, in another aspect, the invention is also directed to a new system consisting of a microfabricated device and an external manifold of electropneumatic reservoirs. One embodiment of the system of the invention (designed for capillary electrophoresis with a liquid junction interface) is shown in Fig. 4.

Referring to Fig. 4, in the system of the invention, a plastic manifold 1 of electropneumatic reservoirs, with attached electrodes 2, serves also as a mounting base for microdevice 3. A sample is introduced into the microdevice via a sample inlet 7. The fluid connection between reservoirs in the manifold and the microdevice is facilitated by channel openings 4 on the edge of the microdevice 3, and sealing is achieved by miniature o-rings 5 placed into the grooves 6 of the manifold. This arrangement eliminates the need for drilling holes into the microdevice and can be used universally for practically all microdevice designs with single or multiple channels. The example in Fig. 4 is designed for off-chip transport, via an electrospray interface, and subsequent detection; however, the identical system can be used for any other application of a microfluidic device with on- or off-chip fluorescence, (UV, electrochemical, conductivity, etc.) detection. If electric connection is required without liquid transport between the manifold and the microdevice, a piece of a membrane can be placed between

the microdevice and the o-ring. Such a membrane can also serve as a selective barrier for ions of desired properties - size, affinity, etc. or can be used for sample preconcentration and/or delivery of chemical reagents Such an arrangement is shown in Fig. 5.

Fig. 5 shows a channel 1 on the microdevice separated from the plastic manifold 2 by a membrane 3. The leak-free sealing is facilitated by an o-ring 4 placeg between membrane 3 and the plastic manifold 2. If the membrane is permeable to the solvent, it can also be used for osmotic or electroosmotic pumping of the liquid from (to) the external reservoir to (from) the channel on the microdevice. The osmotic pumping would be initiated by a difference in the chemical potentials of the liquids in the microdevice and the external reservoirs. For electroosmotic pumping, the membrane should have a surface charge (ion exchange membrane). In such a case, the liquid flow can be initiated by application of an electric field on the membrane.

The disclosed device has been successfully applied for analysis of proteins, peptides and chemical libraries. An example of the analysis of the protein tryptic digests is in Fig. 6, which shows an example of eight consecutive CE separations of protein digests with the microfabricated system described in this disclosure. The separation channel was washed after each analysis. Detection was by ESI/MS.

The described design will be useful for all applications where the capacity of the on-chip reservoirs is insufficient for practical use and where pneumatic fluid control is required. Such applications

include, but are not limited to, sample fraction collection, drug screening, flow cytometry, protein and DNA analysis.

The following examples are presented to illustrate the advantages of the present invention. These examples are not intended in any way otherwise to limit the scope of the invention.

EXAMPLE I

High Throughput Microtiter Plate Electrospray Mass Spectrometry Infusion System

The described system (Fig. 1) is based on the coupling of commercially available microtiter plates (96/384/1536 etc.) to a subatmospheric ESI-interface, allowing for sample introduction into the mass spectrometer without the use of an external pressure source. The samples are stored in a microtiter plate, arranged vertically on a three dimensional translation stage in front of the sampling interface. The infusion system was successfully applied to the purity and identity control of combinatorial libraries. Sample turnaround times of 5-10 sec/sample, with 100 nL sample consumption per analysis, were achieved. Applications in combinatorial chemistry, such as mass identity analysis for HPLC fractions, as well as raw synthesis products are shown.

Instrumentation

The infusion system consisted of a motorized XYZ-stage that positioned a sample containing microtiter plate in front of a subatmospheric microsprayer. Samples were introduced directly into the microsprayer, formed

by a short piece of fused silica capillary (7 cm, 27m ID, 365 m OD) obtained from Polymicro Technologies (Phoenix, AZ). The spray tip was etched by concentrated HF solution. The motorized XYZ-stage was assembled from 3 individual Posi-drive stages (Deltron, Bethel, CT), connected to NEMA size 23 stepper motors (AMSI Corp., Smithtown, NY) and high speed chopper drivers for the stepper motors (AMSI Corp.).

The subatmospheric ESI-microspray interface and washing device were designed in house. The wash device was machined from Teflon, and attached with an aluminum extension arm to the Z stage. High voltage was achieved by contact through a stainless steel electrode in the wash solution. Vacuum was controlled with two pneumatic switches (NV J 114,SMC, Indianapolis, IN). A LabView (National Instrument Corp., Austin, TX) software program controlled automatic as well as manual operations of translation stage and pneumatic switches.

Mass Spectrometry

Electrospray mass spectra were acquired on a Finnigan LCQ ion trap mass spectrometer (San Jose, CA), equipped with an ESI source. The conventional ESI assembly was replaced with the subatmospheric microspray interface for sample delivery placed in the axis 2-3 mm from the heated capillary (held at 200 C). Solutions were sprayed with a potential of +2 kV. Data acquisition was performed in the full scan mode (m/z: 200-2000), and in the MS/MS mode for cross contamination studies.

Materials

Angiotensin standards and verapamil were purchased from Sigma (St Louis, MO). HPLC grade methanol and acetonitrile were obtained from Fisher and glacial acetic acid was obtained from J.T. Baker (Phillipsburg, PA). Deionized water was generated in house with Millipore Alpha Q water purifier (Millipore, Medford, MA). Combinatorial HPLC fractions from ArQule (Woburn, MA) as well as raw synthesis samples from CombiChem (San Diego, CA) were received in deep well 96 microtiter plates.

Sample Preparations

Angiotensin standards: A stock solution of 1 mg/ml angiotensin in water was made up and diluted to a final concentration of 0.05 mg/mL in 50% (v/v) methanol, 49.5 % (v/v) water and 0.5% (v/v) acetic acid (v/v/v).

The samples obtained from ArQule were prepared as follows: 96 well plate: 10 L of original sample (~ 80% (v/v) acetonitrile/ ~ 20% (v/v) H_2O/ ~1-2% (v/v) TFA) diluted with 10 L of 100 % (v/v) acetonitrile and transferred by an 8 channel pipette into a conical shaped PCR plate. The samples obtained from CombiChem were prepared as follows: 96 well plate: 1 L of original sample (100 % (v/v) DMSO) were diluted with 99 L of 75% (v/v) acetonitrile/ 24% (v/v) H_2O/ 1% (v/v) acetic acid and transferred by an 8 channel pipette into a conical shaped PCR plate.

Instrument Design

The system was designed to eliminate all possible sources of dead volume present in conventional FIA

autosampler systems, e.g. transfer lines from microtiter plate to injector loop and from injector loop to MS. The design was based on a subatmospheric ESI-microspray interface, which operated unassisted, e.g. without external pressure source or sheath liquid. The interface was similar to the ESI interface developed previously for CE-MS analysis. The device used consisted of a short (~7 cm) narrow bore (27m ID) fused silica capillary, incorporated in Plexiglas housing. The capillary, which represented spray tip as well as transfer capillary, held a volume of ~ 70 nL, and the Plexiglas housing was cut to fit gas tight over the orifice of the Finnigan LCQ ion trap MS. The flow was generated through the pressure difference between the subatmospheric spray chamber (outlet) and the atmospheric pressure at the inlet of the capillary. Two computer controlled pneumatic pressure switches were used for rapid pressure changes in the ESI-chamber. Both switches could be individually actuated, resulting in a zero flow option and two flow rates options (Figure 2.3). The high flow rate, ~1μL/min, was used for flushing of the ESI-capillary, and the low flow rate of < 250 nL/min was used for sample analysis. In order to maintain the transfer/spray capillary as short as possible, the sample containing the microtiter well plate was placed vertically in front of the ESI interface. Furthermore, to eliminate unnecessary sample transfer steps, the system was designed to support standardized microtiter plates commonly used in sample synthesis and processing.

One concern with arranging the microtiter plate vertically was maintaining the sample liquid inside the

sample well (i.e., no sample flow out of the well). As a test, the wells were filled with 100 L of the respective spray/sample solution and vertically arranged. The plates, covered with a thin PVC sealing film in order to prevent evaporation, were monitored for possible sample leakage over the period of one hour. The results of this test showed that common spray solutions, such as solutions of 75 % (v/v) methanol as well as pure acetonitrile, showed no leakage.

This experiment demonstrated that for V-bottom 96 well plates the capillary forces and surface tension are sufficiently strong to retain common spray solutions inside the wells. The thin sealing film was also pre-pierced by razor blades before the analysis, as piercing of the film with the sampling capillary could contaminate or plug the capillary. The microtiter well plate was placed in front of the fixed subatmospheric sprayer, in a holder that was casted from epoxy, and then mounted on a computer controlled three-dimensional translation stage.

A major concern in high throughput analysis is cross contamination of the sample flow path from previous samples. To avoid such problems, all parts which were in contact with sample solution, such as the inlet of the capillary, were washed on the inside as well on the outside of the capillary after each run. The wash device, shown in Fig. 3, was machined from a single Teflon block, with a flow-through channel coupled to a syringe pump. An opening was made perpendicular to the flow-through channel, to allow the fixed sampling capillary to operate smoothly through the flow channel.

The washing device was mounted on the Z-translation stage, allowing back and forth movement over the inlet of the capillary.

Sample Analysis

As discussed in the Introduction, two different methods of infusion MS analysis are known: flow injection analysis or direct infusion analysis. The advantage of the instrument described herein is the ability to operate, fully automated, in both modes. Due to the small size of the sampling/spray capillary and the possibility of varying the flow rate (70 nL total volume, flow rate 0 -1000 nL/min), the system could be partially or fully filled, without large sample consumption or slow turnaround times.

For the first experiment, the instrument was first set to run in the direct infusion mode. A 96 well plate was filled with solutions of angiotensin standards and infused directly into the ESI capillary. High sample flow was used for complete capillary filling. During this step, no signal was monitored, as the flow was too high to generate a stable unassisted micro-electrospray. After complete filling of the capillary, the flow was decreased to 250 nL/min, and the electrospray was stabilized within less than 1 second, yielding analyte signal.

After complete filling, the capillary inlet entered the washing device while the translation stage moved to the next well. During this operation, data was acquired, and the flow of wash solution was maintained at 250 nL/min. The plug of infused wash liquid represented

about 30 nL (1/3 of the total capillary volume), and since the capillary was flushed with the new sample before the next analysis, no cross contamination of the mass spectra was observed. With ~ 2.5 sec/sample used for data acquisition, the resulting duty cycle was ~ 30 %, with a total sample consumption of about 100 nL. In the present system, the signal could be observed as long as desired, spending short time to acquire strong signals or a longer time to acquire weak signals of low concentration samples. Thus, to increase duty cycle, the instrument had been set to run at a turnaround time of 10 sec/sample. This timing resulted in 12 scans per sample and an increase in duty cycle to 40%.

Direct infusion consumed ~100 nL of sample per analysis, which was on the order of a micro FIA analysis. To demonstrate the instruments capability of the instrument to operate with even lower sample consumption (~ 20nL sample), a partial capillary filling method was developed. The flow rate for sample infusion as well as wash solution infusion was ~ 250 nL/min. After plug injection, the capillary inlet entered the washing device, and the translation stage moved to the next sample well. During this operation, data was acquired, and the flow of the wash solution was maintained at 250 nL/min flowrate. The ESI-capillary was washed with about 50 nL of wash solution (~2/3 of the total capillary volume).

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Weinmann, W. and M. Svoboda, *J Anal Toxicol*,22 (1998). p. 319-28.

While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only by the appended claims and equivalents thereof.

CLAIMS

What is claimed is:

1. An automated liquid handling infusion system comprising

a microscale sample holder, said holder configured for holding multiple samples, said holder being situated in conjunction with a positioning system capable of automated operation;

a sample infusion capillary, said capillary having an inlet end and an outlet end, said inlet end of said capillary being in alignment with one of said multiple samples in said sample holder; and

a source of positive or negative pressure for applying said positive or negative pressure across said sample infusion capillary.

2. The liquid handling infusion system of claim 1, wherein said microscale sample holder is a microtiter well plate.

3. The liquid handling infusion system of claim 1, wherein said positioning system is a computer controlled translation stage.

4. The liquid handling infusion system of claim 1, wherein said sample infusion capillary is 2-200 mm long with internal diameter of 2-200 micrometers.

5. The liquid handling infusion system of claim 1, wherein said sample infusion capillary is a capillary array for parallel infusion of several samples.

6. The liquid handling infusion system of to claim 1, wherein said infusion capillary is configured for washing between analyses by a washing device.

7. The liquid handling infusion system of claim 6, wherein a washing reservoir of said washing device is attached to the positioning system.

8. The liquid handling infusion system of claim 6, wherein a washing reservoir of said washing device is a part of the sample holder.

9. The liquid handling infusion system of claim 1, further comprising a chamber coupled to the outlet end of said sample infusion capillary.

10. The liquid handling infusion system of claim 9, wherein the pressure in the chamber can be regulated.

11. The liquid handling infusion system of claim 9, wherein the chamber is an electrospray chamber.

12. The liquid handling infusion system of claim 9, wherein the chamber is a chamber for sample deposition on a movable surface.

13. The liquid handling infusion system of claim 1, wherein said sample infusion capillary further comprises a separation capillary.

14. The liquid handling infusion system of claim 1, wherein said sample infusion capillary is a channel in a microfluidic device.

15. The liquid handling infusion system of claim 14, wherein said channel is open.

16. The liquid handling infusion system of claim 14 where the said channel is filled with an adsorbant packing.

17. A liquid handling reservoir system comprising

a microfluidic device, said device comprising multiple microfabricated channels, each said channel having an inlet end;

a detachable reservoir manifold external to said device, said reservoir having at least one opening for fluid flow into and out of said reservoir, said reservoir positioned so that said reservoir opening is proximate to an inlet end of a microfabricated channel in said device for fluid flow between said reservoir and said channel; and

a source of positive or negative pressure for applying said positive or negative pressure across said system.

18. The reservoir system of claim 17, wherein the volume of the reservoirs is in the range of 10 to 10,000 microliters.

19. The reservoir system of claim 17, wherein the detachable reservoir manifold provides both pneumatic and electrokinetic sample introduction.

20. The reservoir system of claim 17, wherein the detachable reservoir manifold allows washing of the channels on the microdevice.

21. The reservoir system of claim 17, wherein the reservoirs of the manifold are separated from the microfluidic device by a semipermeable membrane.

22. The reservoir system of claim 17, wherein the membrane is used for electroosmotic pumping of the liquid to or from the manifold to the microfluidic device.

23. The reservoir system of claim 17, wherein the membrane is used for sample preconcentration.

24. The reservoir system of claim 17, wherein the membrane contains an immobilized reagent for sample modification.

25. The reservoir system of claim 17, wherein the reagent is an enzyme.

26. The reservoir system of claim 17, wherein the reagent is forming a fluorescence label on the analyzed molecules.

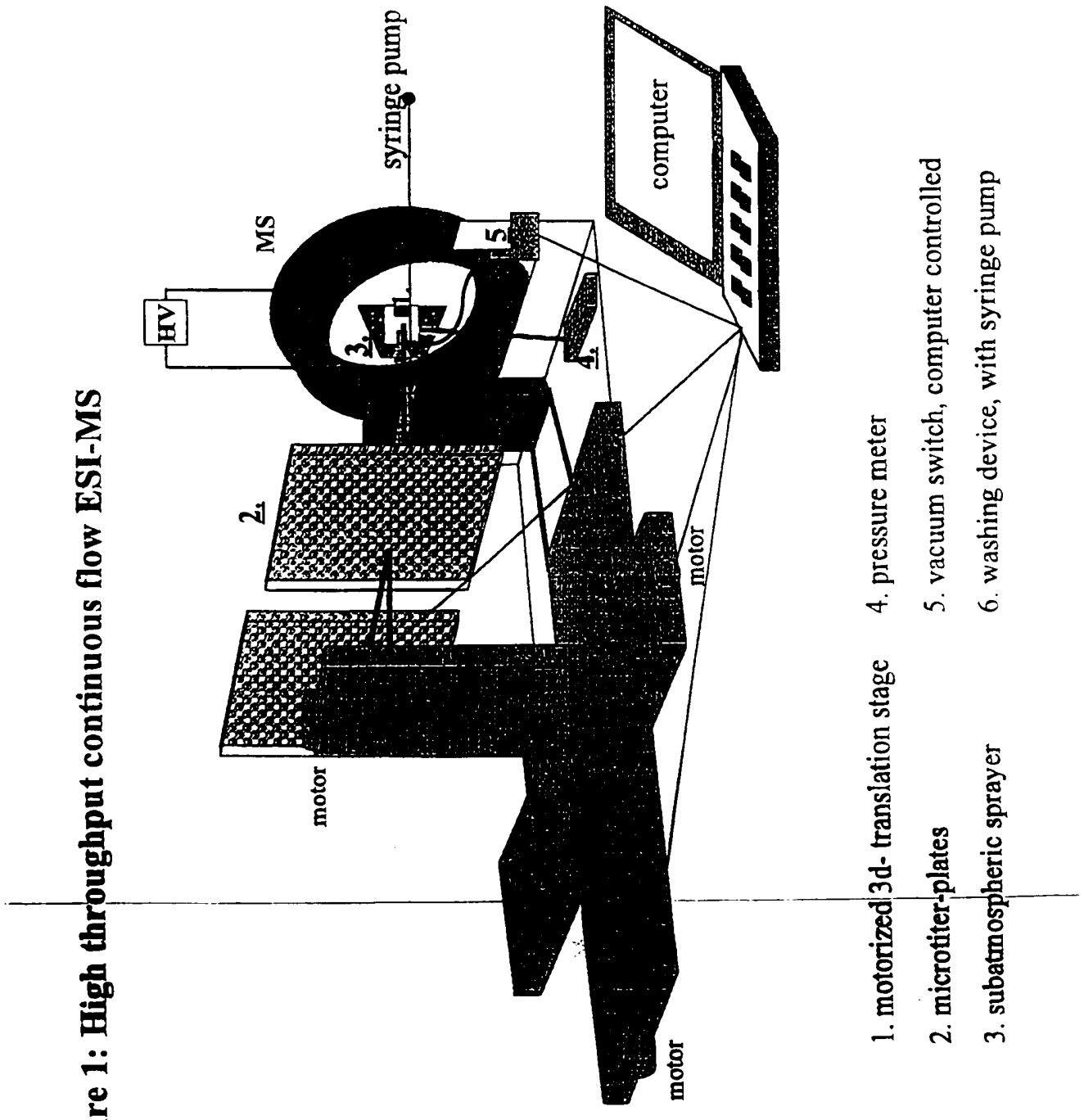


Figure 1: High throughput continuous flow ESI-MS

1. motorized 3d- translation stage
2. microtiter-plates
3. subatmospheric sprayer
4. pressure meter
5. vacuum switch, computer controlled
6. washing device, with syringe pump

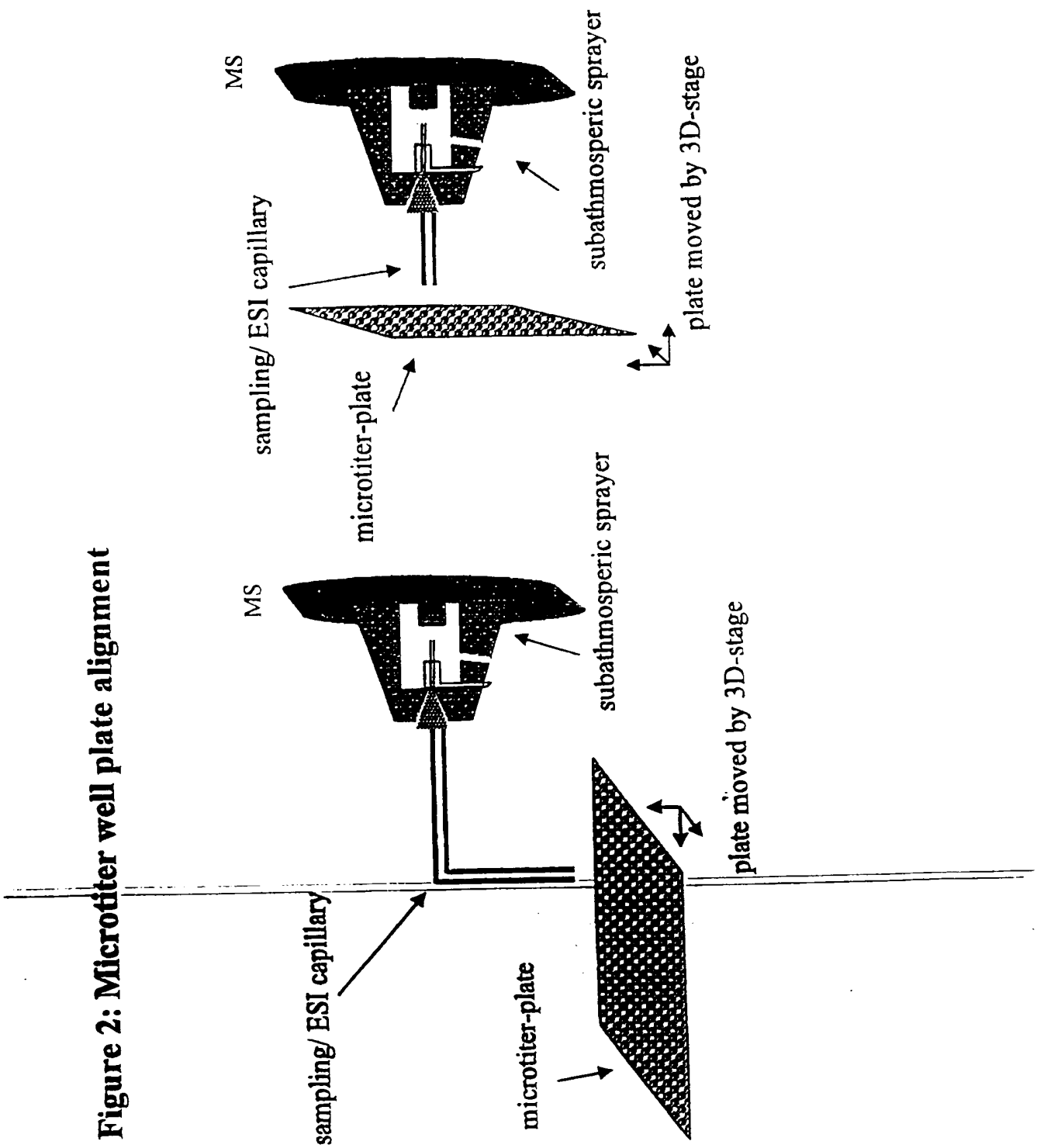
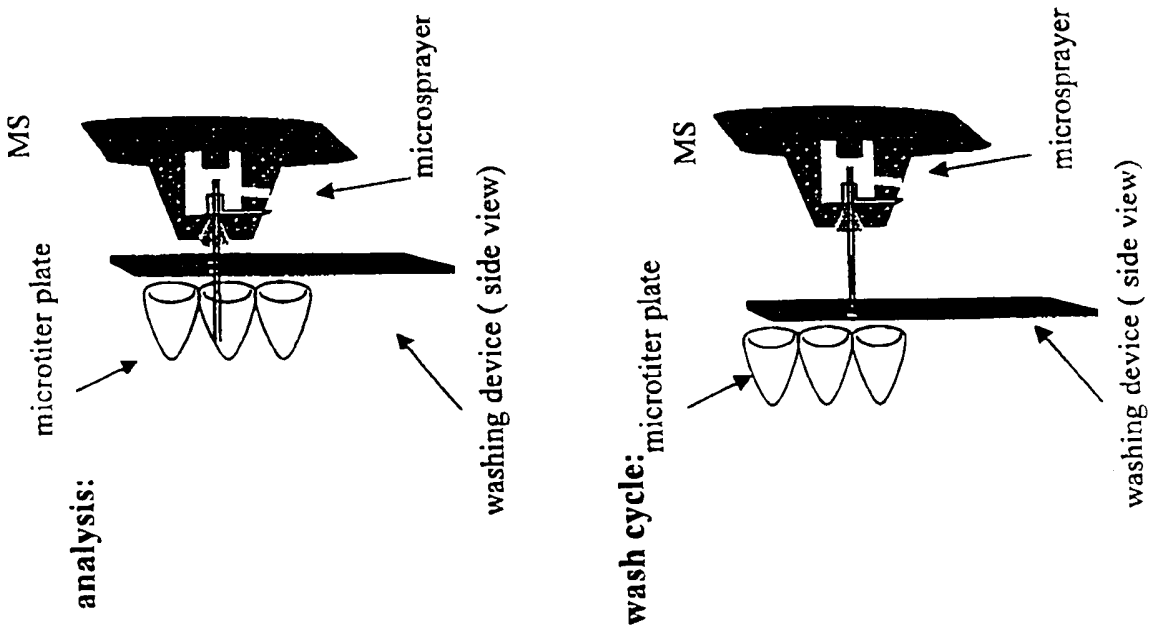


Figure 2: Microtiter well plate alignment



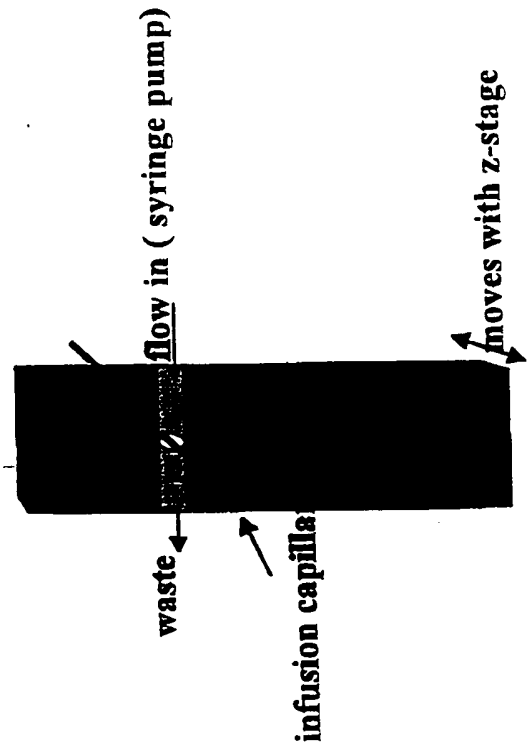


Figure 3: Flow Through Wash Device

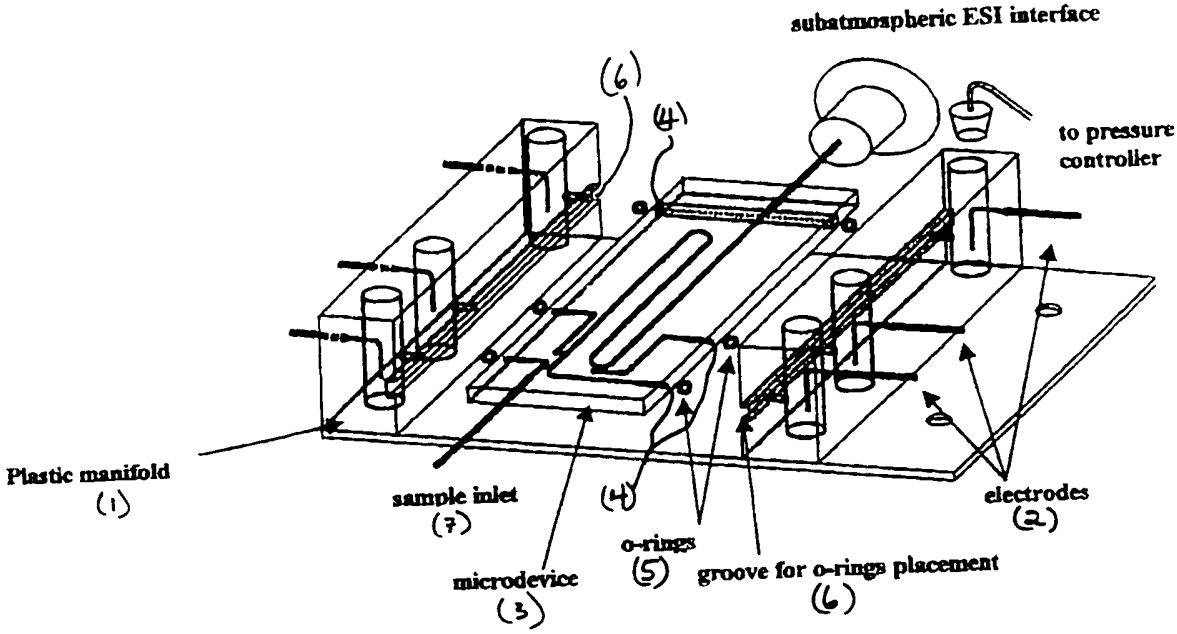

subatmospheric ESI interface
(6)
(4)
to pressure controller
Plastic manifold
(1)
sample inlet
(7)
(4)
o-rings
(5)
groove for o-rings placement
(6)
microdevice
(3)
electrodes
(2)

Fig. 4

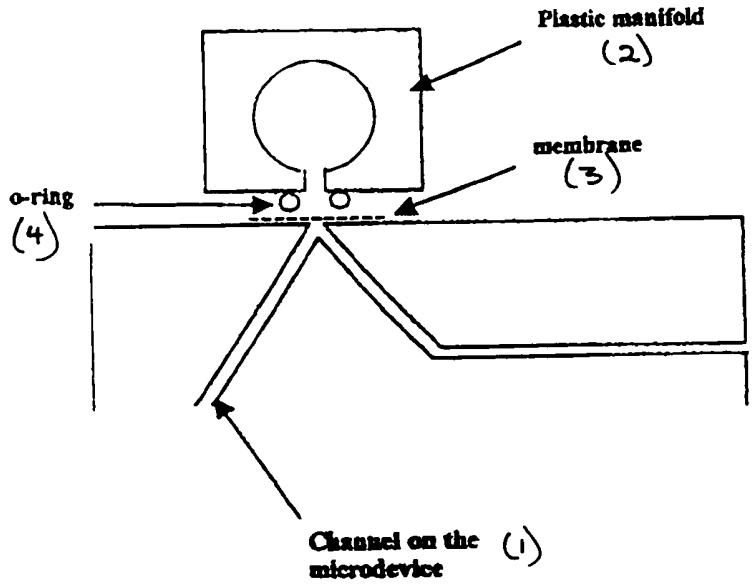

Plastic manifold
(2)
membrane
(3)
o-ring
(4)
Channel on the microdevice (1)

Fig. 5

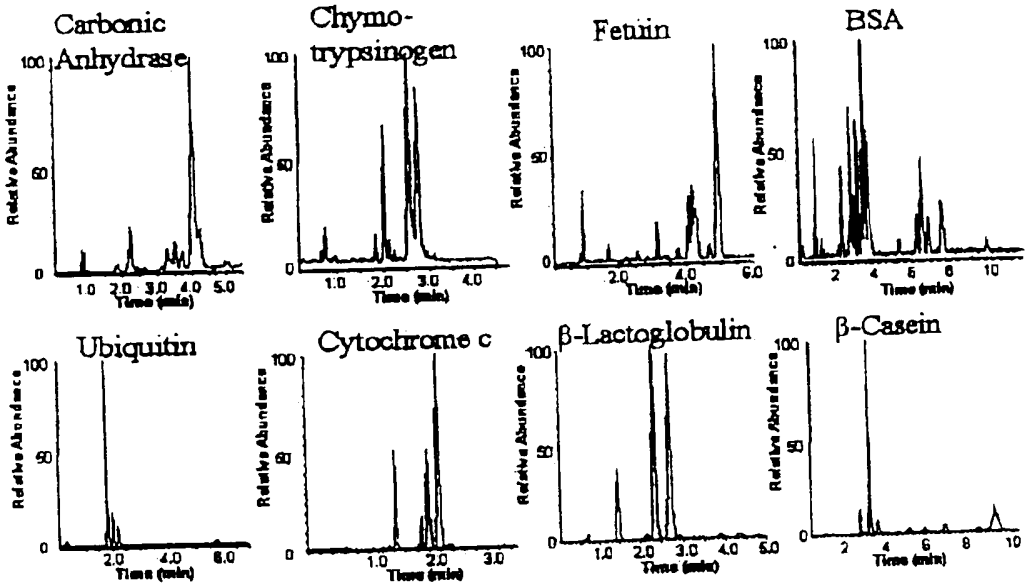

Carbonic Anhydrase
Chymo-trypsinogen
Ferritin
BSA
Ubiquitin
Cytochrome c
β-Lactoglobulin
β-Casein
Relative Abundance
Time (min)

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/09480

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 21/00, 31/00, 33/00; B01L 3/00, 3/02

US CL :422/63, 81, 99, 100

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/63-67, 68.1, 81, 99-104; 436/180; 73/863.01, 863.31, 864.01, 864.11, 864.12, 864.15

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST keyword search on terms: MALDI, liquid dispensing, capillary, microplate, microfluidic, and microchannel

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	US 6,024,925 A (LITTLE et al) 15 February 2000, Figures 1 and 4, columns 6-8.	1,2,4,5,9,10, 12
Y	US 5,872,010 A (KARGER et al) 16 February 1999, Figures 6A and 6B, column 6, lines 10-67, col. 7, l. 1-65.	1 , 3 , 4 , 9 - 12,14,15,17,18
Y	WO 99/14368 A (ADOURIAN et al) 25 March 1999, Figures 1 and 4, page 15, lines 14-38, pages 16-20 all.	1,2,4-6,9,14-16
Y	US 5,498,545 A (VESTAL) 12 March 1996, Figure 8, columns 4-6.	1,2,9,10,12
A	US 5,580,434 A (ROBOTTI et al), 03 December 1996, entire document.	

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
26 JUNE 2000

Date of mailing of the international search report
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Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
DWAYNE K. HANDY
Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/09480

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,969,350 A (KERLEY et al) 19 October 1999, entire document.	

Form PCT/ISA/210 (continuation of second sheet) (July 1998)*

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(71) Applicant *(for all designated States except US)*: **NORTHEASTERN UNIVERSITY** [US/US]; 360 Huntington Avenue, Boston, MA 02115 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KARGER, Barry, L.** [US/US]; 62 Deborah Road, Newton, MA 02159 (US). **FELTEN, Chantal** [LU/US]; 140 West Canton Street, Boston, MA 02118 (US). **FORET, Frantisek** [CZ/US]; 525 Highland Avenue, #40, Malden, MA 02148 (US). **ZHANG, Bailin** [CN/US]; Apartment #1, 104 Hemenway Street, Boston, MA 02115 (US).

(74) Agents: **HEINE, Holliday, C.** et al.; Weingarten, Schurgin, Gagnebin & Hayes LLP, Ten Post Office Square, Boston, MA 02109 (US).

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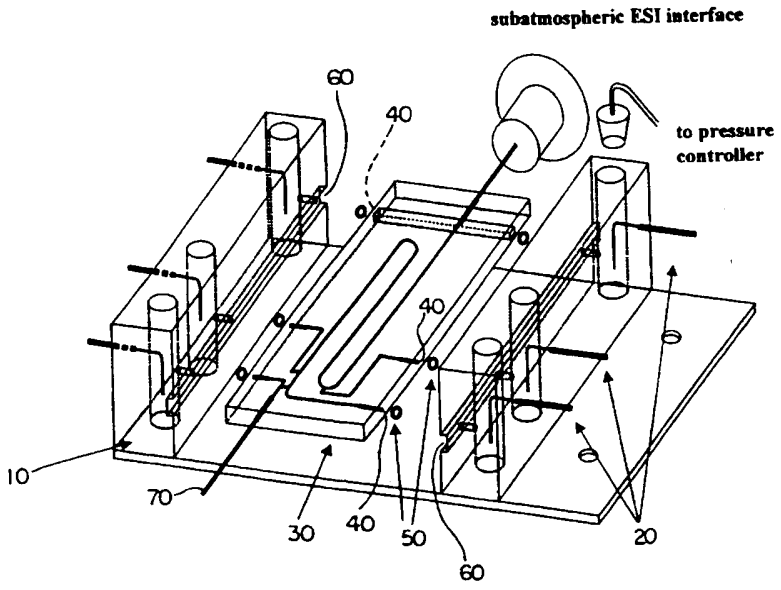
(54) Title: SYSTEM AND METHOD FOR HIGH THROUGHPUT MASS SPECTROMETRIC ANALYSIS



(57) Abstract: Disclosed is a liquid handling reservoir system comprising a microfluidic device (3), the device comprising multiple microfabricated channels (4), each channel having an inlet end (7); a detachable reservoir manifold (1) external to the device, the reservoir (1) having at least one opening for fluid flow into and out of the reservoir (1), the reservoir (1) being positioned so that the reservoir opening is proximate to an inlet end (7) of a microfabricated channel (4) in the device for fluid flow between the reservoir (1) and the channel (4).

WO 00/62039 A1

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE OF THE INVENTION

SYSTEM AND METHOD FOR HIGH THROUGHPUT MASS SPECTROMETRIC ANALYSIS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the priority of U.S. Provisional Patent Application No. 60/128,509 filed, April 9, 1999 entitled SYSTEM AND METHOD FOR HIGH THROUGHPUT MASS SPECTROMETRIC ANALYSIS, the whole of which is hereby incorporated by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

BACKGROUND OF THE INVENTION

Recent developments in microfabrication techniques have permitted the integration of microminiature tools for biochemical analysis within a tiny device. Complete chemical processing systems, e.g., reaction chambers, separation capillaries and their associated electrode reservoirs, as well as certain types of detectors, can be consolidated on a microchip of, e.g., a glass or fused silica. Such "labs-on-a-chip," in principle, permit effective utilization and manipulation of minute quantities of material. Systems have been developed that permit the efficient transfer of nanoliter quantities or other small quantities of a fluid sample from the spatially concentrated environment of a microscale device

to "off-chip" analytical or collection devices without an increase in sample volume. (See, U.S. Patent No. 5,872,010, the whole of which is hereby incorporated by reference herein.) However, methods for moving small quantities of samples from a sample vial or holder onto a chip or directly to an analytical device without either evaporation or dilution are less well developed.

SUMMARY OF THE INVENTION

In one aspect the invention is directed to a liquid handling infusion system comprising a microscale sample holder configured for holding multiple samples, the holder being situated in conjunction with a positioning system; a sample infusion capillary having an inlet end and an outlet end, the inlet end of said capillary being in alignment with one of the multiple samples in the sample holder; and a source of positive or negative pressure for applying the positive or negative pressure across the sample infusion capillary. In other embodiments, the infusion system further comprises an ESI chamber coupled to the outlet end of the sample infusion capillary, the sample infusion capillary further comprises a separation capillary, or the sample infusion capillary is a channel (open or closed) in a microfluidic device.

In another aspect the invention is directed to a liquid handling reservoir system comprising a microfluidic device, the device comprising multiple microfabricated channels, each channel having an inlet end; a detachable reservoir manifold external to the device, the reservoir having at least one opening for

fluid flow into and out of the reservoir, the reservoir being positioned so that the reservoir opening is proximate to an inlet end of a microfabricated channel in the device for fluid flow between the reservoir and the channel.

The systems of the invention, alone or in combination, are suitable for moving small quantities of samples from a sample vial or holder onto a chip or directly to an analytical device, without either evaporation or dilution, and for providing for rapid and automated washing of the infusion capillaries and/or the complete system.

The samples may be transfered for electrospray-mass spectrometry analysis (ESI/MS), for atmospheric pressure-chemical ionization mass spectrometry analysis (APCI/MS), for matrix assisted laser desorption ionization mass spectrometry (MALDI/MS), for nuclear magnetic resonance analysis (NMR), for pneumatically or ultrasonically assisted spray sample handling, for transfer to an off-chip detection system, such as electrochemistry, conductivity or laser induced fluorescence, or for collection of specific fractions, e.g., in collection capillaries or on collection membranes.

The channels of any microdevice used may be arrayed in any format that allows for sequential or simultaneous processing of liquid samples.

In any embodiment, each channel may include electrical contacts, so that an electric circuit path can be established along the channel. For example, one electrical contact can be on the entrance side of a channel and another electrical contact can be on the exit

side. In an alternative arrangement, an electric circuit can be completed by an external contact, beyond the exit end of the channel. For example, if the exit port of a channel is used as an electrospray source for a mass spectrometer, the mass spectrometer sampling orifice can serve as the counter electrode. Samples can be transferred off chip for subsequent analysis by switching the electric current sequentially to each channel on the chip.

Samples can be introduced into a channel on the microscale device of the invention by a variety of methods, e.g., by pressure, electrokinetic injection, or other technique, and an electrical current and/or pressure drop can then be applied to cause the sample components to migrate along the channel. The channels may function only for fluid transfer, e.g., to a mass spectrometer, or the channels can serve as environments for various types of sample manipulations, e.g., for micropreparative or analytical operations, such as capillary electrophoresis (CE), chromatography or the polymerase chain reaction (PCR), or for carrying out any type of sample chemistry. The channels may be filled with membrane or packing material to effectuate preconcentration or enrichment of samples or for other treatment steps, such as desalting. Furthermore, other modification of sample components, e.g., by enzymes that are covalently bound to the walls of a channel or are free in a channel, are possible. Packing material may be bound to the walls of the channels or may include other components, such as magnetic particles, so that when a magnetic field is applied, the magnetic particles retain

the packing material in place. The magnetic particles can also be used for efficient mixing of fluids inside the channels, using an external magnetic field. A micromachined filter or other stationary structure may also be employed to hold packing material in place. Alternatively, stationary structures can be micromachined, cast or otherwise formed in the surface of a channel to provide a high surface area which can substitute for packing material.

A sample can be introduced into a channel in a short starting zone or can fill the whole channel completely. Filling only a small part of the channel with the sample is preferable when an on-chip separation of sample components is to be carried out, such as electrophoresis or chromatography. Filling the whole channel with the sample may be advantageous in cases when off-chip analysis requires extended sample outflow, such as sample infusion/electrospray ionization for structure analysis by mass spectrometry.

In many cases a liquid flow may be required to transport the analytes in a sample into a specific channel, or along the length of the channel, or out of the channel via an exit port. Therefore, to assist in the required fluid transfer, a pumping device may be incorporated into or associated with the microscale device of the invention. For example, a heating element can be used to cause thermal expansion, which will effectuate sample liquid movement, or a heating element can be used to generate a micro bubble, the expansion of which causes the sample to travel in the channel. Other options may include pumping by the pressure of a gas or

gases generated by on-chip electrolysis. Flow can be also generated by application of a pressure drop along a channel or by electroosmosis inside a channel.

As samples move to the end of a channel, they can be subjected to detection or analysis at a site external to the microscale device of the invention by a variety of techniques, including mass spectroscopy, nuclear magnetic resonance, laser induced fluorescence, ultraviolet detection, electrochemical detection, or the like. The end of each channel may include a tip configured to facilitate transfer of the sample volume.

The invention may be used in a fluid sheath (e.g., liquid or gas) or sheathless mode depending on the type of analysis required and the size of the sample exiting a channel. In a sheathless arrangement, the exit port is formed at the end of the channel. When a liquid sheath is required (e.g., for the addition of a liquid, a chemical and/or a standard prior to electrospray or to provide electric connection via the sheath fluid), an exit port can be created at the merge point of two channels, one supplying the sample and the other the sheath liquid. Selective analysis of analytes in both the cationic and anionic modes can be performed easily by rapid switching of the polarity of the electric field.

Different sized channels may be employed on the same microscale device. For example, larger channels may be used for cleanup operations, and smaller channels may be used for processing operations. Moreover, other operations can be performed in other regions of the device, such as chemical processing, separation, isolation or detection of a sample or a component of the

sample, prior to sample loading in a channel. Thus, it is possible to carry out sample chemistries or to conduct micropreparative and analytical operations on both a starting sample and its separated components within the device of the invention, prior to transfer of the sample or its components off chip for further analysis or collection. Additionally, detection of a sample may be carried out on the microdevice itself, e.g., by a fiber optic detection system, which can provide complementary control information for off-chip analysis and detection, or by any other suitable detector such as laser induced fluorescence, conductivity and/or electrochemical detector.

Buffer reservoirs, reaction chambers, sample reservoirs, and detection cells may also be fabricated along with each individual channel. More complex structures can be created by stacking or otherwise assembling two or more microfabricated devices. In addition, individual instrument blocks such as sample reservoirs, pretreatment or separation channels, and exit ports can be micromachined separately and combined into one complete system in much the same way as hybrid integrated circuits in electronics are formed. Microfabrication techniques are precise and will allow for a high degree of reproducibility of selected channel and exit port shapes and dimensions.

The microscale fluid handling system of the invention permits more efficient use of powerful analytical devices, such as the mass spectrometer, than is currently possible. In addition, the system of the invention can be manufactured as a disposable device that

is suitable for cost effective automation of the analysis of a large number of samples. Using this micromachined approach, high throughput analysis by mass spectrometry would be possible. In addition, handling of small volumes and quantities of samples would be facilitated, and consumption of valuable samples and reagents would be reduced. Applications include any laboratory analysis methods, especially where high throughput and minimization of cross-contamination are desirable, such as screening and diagnostic methods, and such other analytic methods as pharmacokinetics where fresh columns are required for each run.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a high throughput continuous flow electrospray interface system for mass spectrometry, according to the invention;

Fig. 2 shows the alignment of a microtiter well plate sample holder in the system of the invention;

Fig. 3 shows a flow through washing device according to the invention;

Fig. 4 shows a liquid handling reservoir system according to the invention;

Fig. 5 shows a detail of the sustem of Fig. 4 in which a detachable manifold is separated from a channel on the associated microdevice by a porous membrane; and

Fig. 6 shows the results of experiments carried out using the sustem of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

The current acceleration of pharmaceutical research directed to identifying new drug candidates and the related increase in the number of pharmacokinetic studies require suitable techniques for high throughput analysis. In addition to a number of assays based on optical measurement, mass spectrometry is gaining in importance for pharmacokinetic studies as this technique can provide sensitivity combined with speed, mass selectivity and structural information. High throughput electrospray/MS systems predominantly utilize the flow injection mode of operation. In this injection method, a zone of sample is introduced into a stream of a carrying fluid (fluid sheath) for transport into the electrospray source for ionization and analysis. Typically, a modified liquid chromatography fluidic system is used for the task. Since a flow injection system consists of a number of relatively long connection tubes, relatively large sample volumes are required for analysis. Frequently, more than 10 µL of sample is consumed per analysis, although only a fraction of this amount is required to be electrosprayed to generate useful information. One analysis cycle consists of system loop filing, injection/analysis and washing. After the analysis, the whole fluidic system must be washed to prevent contamination of the next

injected sample. Typically, analysis cycles of 40-60 sec can be achieved, but the time for actual analysis and data collection will only be a few seconds, as most of the cycle time will be used for system flushing. Thus, the main disadvantage of the flow injection system are large sample volume consumed; low duty cycle as a large part of the analysis time is lost during the system washing; time for analysis limited by sample loop size; and limited use of low volume microtiter well plates.

The infusion system of the invention, schematically shown in Fig. 1, is based on the subatmospheric electrospray interface described in pending U.S. Application No. 08/784,400, the whole of which is hereby incorporated by reference herein. The system of the invention is useful, e.g., for high throughput ESI/MS analysis of small (submicroliter) sample volumes without the abov-mentioned limitations. Referring to Fig. 1, the system of the invention consists of a microtiter well plate 2, vertically arranged on an XYZ stage 1 and interfaced to an ion trap mass spectrometer by a fixed subatmospheric ESI-microsprayer 4. The infusion flow is achieved through the application of a vacuum inside the spray chamber. A simple flow through wash reservoir 3 is inserted between the ESI-interface and the microtiter plate, allowing for external as well as internal washing of the infusion capillary. Wash reservoir 3, machined from a Teflon block, is connected by Teflon tubing to a syringe pump containing the appropriate wash solution. The infusion capillary passes through the opening of the wash device reservoir, thus allowing back and forth

movement of the wash device over the inlet of the infusion capillary.

The system uses one capillary (e.g, 10 mm - 10 cm, with an id of 10-100 μm) for both the sample aspiration and electrospray ionization. Alternatively, the electrospray chamber can be operated at atmospheric pressure, and the sample flow generated by pressurizing the sample inlet side. As shown in Fig. 2, by positioning the microtiter well plate vertically, the length of the sampling/ESI capillary can be significantly reduced, resulting in lower sample consumption. Once this capillary is dipped into the sample, the lower pressure in the ESI chamber will cause sample flow. The ESI capillary is connected to a high voltage power supply that results in electrospray of the sample at the tip. The capillary remains in the sample vial and continuously feeds sample tot he mass spectrometer until sufficient information is obtained, and the stage then moves to the next sample position for the next analysis. To avoid contamination of the next sample, the ESI capillary is washed in a flow through reservoir during the sample position change as shown in Figure 3. A computer-controlled valve can be used to adjust the pressure in the ESI chamber to control the sample and washing fluid flows, respectively.

Since the internal volume of the sample/ESI capillary is only a few nL (10-200), very small sample amounts are consumed. At the same time, the useful signal can be observed as long as required, since the sample is not carried into the electrospray as a zone in the mobile phase, but delivered as a continuous flow

directly from the sample vial. Very high duty cycle (over 80%) can be obtained and sample throughput of more than one sample per 5 seconds can easily be achieved. Since the useful MS signal can be observed as long as the ESI capillary is in the sample well, a simple control software can be used to decide if MS/MS analysis is required. There is no need for a new sample injection or flow programming which would be required in a conventional flow injection system.

As a logical extension of the technique, one can also envision that the electrospray capillary be substituted by a miniaturized separation system. For example, a separation column or a microdevice (lab-on-a-chip) suitable for sample preparation and separation (CE, CEC, LC, IEF) can be used in place of the sampling capillary for multi-dimensional analysis of complex mixtures.

The practical use of microfabricated-microfluidic devices requires reservoirs for storage of chemical reagents, electrolytes and samples to be analyzed. The devices currently under development typically integrate such reservoirs directly on the microfabricated device (typically made of glass or plastic), mostly in the form of holes drilled into the surface of the microdevice. Such a design is suitable only for disposable microdevices since such reservoirs have limited volume and cannot be easily replenished after the analysis. At the same time, the fabrication procedures (drilling and gluing) increase the cost of the microdevice.

For devices intended for repetitive analyses or handling of different samples, the volume of the on-chip

reservoirs would likely not be sufficient. Additionally, the small volume of the on-chip reservoirs may suffer from adverse effects of evaporation and electrolysis when electrokinetic transport is used. Thus, in another aspect, the invention is also directed to a new system consisting of a microfabricated device and an external manifold of electropneumatic reservoirs. One embodiment of the system of the invention (designed for capillary electrophoresis with a liquid junction interface) is shown in Fig. 4.

Referring to Fig. 4, in the system of the invention, a plastic manifold 1 of electropneumatic reservoirs, with attached electrodes 2, serves also as a mounting base for microdevice 3. A sample is introduced into the microdevice via a sample inlet 7. The fluid connection between reservoirs in the manifold and the microdevice is facilitated by channel openings 4 on the edge of the microdevice 3, and sealing is achieved by miniature o-rings 5 placed into the grooves 6 of the manifold. This arrangement eliminates the need for drilling holes into the microdevice and can be used universally for practically all microdevice designs with single or multiple channels. The example in Fig. 4 is designed for off-chip transport, via an electrospray interface, and subsequent detection; however, the identical system can be used for any other application of a microfluidic device with on- or off-chip fluorescence, (UV, electrochemical, conductivity, etc.) detection. If electric connection is required without liquid transport between the manifold and the microdevice, a piece of a membrane can be placed between

the microdevice and the o-ring. Such a membrane can also serve as a selective barrier for ions of desired properties - size, affinity, etc. or can be used for sample preconcentration and/or delivery of chemical reagents Such an arrangement is shown in Fig. 5.

Fig. 5 shows a channel 1 on the microdevice separated from the plastic manifold 2 by a membrane 3. The leak-free sealing is facilitated by an o-ring 4 placeg between membrane 3 and the plastic manifold 2. If the membrane is permeable to the solvent, it can also be used for osmotic or electroosmotic pumping of the liquid from (to) the external reservoir to (from) the channel on the microdevice. The osmotic pumping would be initiated by a difference in the chemical potentials of the liquids in the microdevice and the external reservoirs. For electroosmotic pumping, the membrane should have a surface charge (ion exchange membrane). In such a case, the liquid flow can be initiated by application of an electric field on the membrane.

The disclosed device has been successfully applied for analysis of proteins, peptides and chemical libraries. An example of the analysis of the protein tryptic digests is in Fig. 6, which shows an example of eight consecutive CE separations of protein digests with the microfabricated system described in this disclosure. The separation channel was washed after each analysis. Detection was by ESI/MS.

The described design will be useful for all applications where the capacity of the on-chip reservoirs is insufficient for practical use and where pneumatic fluid control is required. Such applications

include, but are not limited to, sample fraction collection, drug screening, flow cytometry, protein and DNA analysis.

The following examples are presented to illustrate the advantages of the present invention. These examples are not intended in any way otherwise to limit the scope of the invention.

EXAMPLE I

High Throughput Microtiter Plate Electrospray Mass Spectrometry Infusion System

The described system (Fig. 1) is based on the coupling of commercially available microtiter plates (96/384/1536 etc.) to a subatmospheric ESI-interface, allowing for sample introduction into the mass spectrometer without the use of an external pressure source. The samples are stored in a microtiter plate, arranged vertically on a three dimensional translation stage in front of the sampling interface. The infusion system was successfully applied to the purity and identity control of combinatorial libraries. Sample turnaround times of 5-10 sec/sample, with 100 nL sample consumption per analysis, were achieved. Applications in combinatorial chemistry, such as mass identity analysis for HPLC fractions, as well as raw synthesis products are shown.

Instrumentation

The infusion system consisted of a motorized XYZ-stage that positioned a sample containing microtiter plate in front of a subatmospheric microsprayer. Samples were introduced directly into the microsprayer, formed

by a short piece of fused silica capillary (7 cm, 27m ID, 365 m OD) obtained from Polymicro Technologies (Phoenix, AZ). The spray tip was etched by concentrated HF solution. The motorized XYZ-stage was assembled from 3 individual Posi-drive stages (Deltron, Bethel, CT), connected to NEMA size 23 stepper motors (AMSI Corp., Smithtown, NY) and high speed chopper drivers for the stepper motors (AMSI Corp.).

The subatmospheric ESI-microspray interface and washing device were designed in house. The wash device was machined from Teflon, and attached with an aluminum extension arm to the Z stage. High voltage was achieved by contact through a stainless steel electrode in the wash solution. Vacuum was controlled with two pneumatic switches (NV J 114,SMC, Indianapolis, IN). A LabView (National Instrument Corp., Austin, TX) software program controlled automatic as well as manual operations of translation stage and pneumatic switches.

Mass Spectrometry

Electrospray mass spectra were acquired on a Finnigan LCQ ion trap mass spectrometer (San Jose, CA), equipped with an ESI source. The conventional ESI assembly was replaced with the subatmospheric microspray interface for sample delivery placed in the axis 2-3 mm from the heated capillary (held at 200 C). Solutions were sprayed with a potential of +2 kV. Data acquisition was performed in the full scan mode (m/z: 200-2000), and in the MS/MS mode for cross contamination studies.

Materials

Angiotensin standards and verapamil were purchased from Sigma (St Louis, MO). HPLC grade methanol and acetonitrile were obtained from Fisher and glacial acetic acid was obtained from J.T. Baker (Phillipsburg, PA). Deionized water was generated in house with Millipore Alpha Q water purifier (Millipore, Medford, MA). Combinatorial HPLC fractions from ArQule (Woburn, MA) as well as raw synthesis samples from CombiChem (San Diego, CA) were received in deep well 96 microtiter plates.

Sample Preparations

Angiotensin standards: A stock solution of 1 mg/ml angiotensin in water was made up and diluted to a final concentration of 0.05 mg/mL in 50% (v/v) methanol, 49.5 % (v/v) water and 0.5% (v/v) acetic acid (v/v/v).

The samples obtained from ArQule were prepared as follows: 96 well plate: 10 L of original sample (~ 80% (v/v) acetonitrile/ ~ 20% (v/v) H_2O/ ~1-2% (v/v) TFA) diluted with 10 L of 100 % (v/v) acetonitrile and transferred by an 8 channel pipette into a conical shaped PCR plate. The samples obtained from CombiChem were prepared as follows: 96 well plate: 1 L of original sample (100 % (v/v) DMSO) were diluted with 99 L of 75% (v/v) acetonitrile/ 24% (v/v) H_2O/ 1% (v/v) acetic acid and transferred by an 8 channel pipette into a conical shaped PCR plate.

Instrument Design

The system was designed to eliminate all possible sources of dead volume present in conventional FIA

autosampler systems, e.g. transfer lines from microtiter plate to injector loop and from injector loop to MS. The design was based on a subatmospheric ESI-microspray interface, which operated unassisted, e.g. without external pressure source or sheath liquid. The interface was similar to the ESI interface developed previously for CE-MS analysis. The device used consisted of a short (~7 cm) narrow bore (27m ID) fused silica capillary, incorporated in Plexiglas housing. The capillary, which represented spray tip as well as transfer capillary, held a volume of ~ 70 nL, and the Plexiglas housing was cut to fit gas tight over the orifice of the Finnigan LCQ ion trap MS. The flow was generated through the pressure difference between the subatmospheric spray chamber (outlet) and the atmospheric pressure at the inlet of the capillary. Two computer controlled pneumatic pressure switches were used for rapid pressure changes in the ESI-chamber. Both switches could be individually actuated, resulting in a zero flow option and two flow rates options (Figure 2.3). The high flow rate, ~1μL/min, was used for flushing of the ESI-capillary, and the low flow rate of < 250 nL/min was used for sample analysis. In order to maintain the transfer/spray capillary as short as possible, the sample containing the microtiter well plate was placed vertically in front of the ESI interface. Furthermore, to eliminate unnecessary sample transfer steps, the system was designed to support standardized microtiter plates commonly used in sample synthesis and processing.

One concern with arranging the microtiter plate vertically was maintaining the sample liquid inside the

sample well (i.e., no sample flow out of the well). As a test, the wells were filled with 100 L of the respective spray/sample solution and vertically arranged. The plates, covered with a thin PVC sealing film in order to prevent evaporation, were monitored for possible sample leakage over the period of one hour. The results of this test showed that common spray solutions, such as solutions of 75 % (v/v) methanol as well as pure acetonitrile, showed no leakage.

This experiment demonstrated that for V-bottom 96 well plates the capillary forces and surface tension are sufficiently strong to retain common spray solutions inside the wells. The thin sealing film was also pre-pierced by razor blades before the analysis, as piercing of the film with the sampling capillary could contaminate or plug the capillary. The microtiter well plate was placed in front of the fixed subatmospheric sprayer, in a holder that was casted from epoxy, and then mounted on a computer controlled three-dimensional translation stage.

A major concern in high throughput analysis is cross contamination of the sample flow path from previous samples. To avoid such problems, all parts which were in contact with sample solution, such as the inlet of the capillary, were washed on the inside as well on the outside of the capillary after each run. The wash device, shown in Fig. 3, was machined from a single Teflon block, with a flow-through channel coupled to a syringe pump. An opening was made perpendicular to the flow-through channel, to allow the fixed sampling capillary to operate smoothly through the flow channel.

The washing device was mounted on the Z-translation stage, allowing back and forth movement over the inlet of the capillary.

Sample Analysis

As discussed in the Introduction, two different methods of infusion MS analysis are known: flow injection analysis or direct infusion analysis. The advantage of the instrument described herein is the ability to operate, fully automated, in both modes. Due to the small size of the sampling/spray capillary and the possibility of varying the flow rate (70 nL total volume, flow rate 0 -1000 nL/min), the system could be partially or fully filled, without large sample consumption or slow turnaround times.

For the first experiment, the instrument was first set to run in the direct infusion mode. A 96 well plate was filled with solutions of angiotensin standards and infused directly into the ESI capillary. High sample flow was used for complete capillary filling. During this step, no signal was monitored, as the flow was too high to generate a stable unassisted micro-electrospray. After complete filling of the capillary, the flow was decreased to 250 nL/min, and the electrospray was stabilized within less than 1 second, yielding analyte signal.

After complete filling, the capillary inlet entered the washing device while the translation stage moved to the next well. During this operation, data was acquired, and the flow of wash solution was maintained at 250 nL/min. The plug of infused wash liquid represented

about 30 nL (1/3 of the total capillary volume), and since the capillary was flushed with the new sample before the next analysis, no cross contamination of the mass spectra was observed. With ~ 2.5 sec/sample used for data acquisition, the resulting duty cycle was ~ 30 %, with a total sample consumption of about 100 nL. In the present system, the signal could be observed as long as desired, spending short time to acquire strong signals or a longer time to acquire weak signals of low concentration samples. Thus, to increase duty cycle, the instrument had been set to run at a turnaround time of 10 sec/sample. This timing resulted in 12 scans per sample and an increase in duty cycle to 40%.

Direct infusion consumed ~100 nL of sample per analysis, which was on the order of a micro FIA analysis. To demonstrate the instruments capability of the instrument to operate with even lower sample consumption (~ 20nL sample), a partial capillary filling method was developed. The flow rate for sample infusion as well as wash solution infusion was ~ 250 nL/min. After plug injection, the capillary inlet entered the washing device, and the translation stage moved to the next sample well. During this operation, data was acquired, and the flow of the wash solution was maintained at 250 nL/min flowrate. The ESI-capillary was washed with about 50 nL of wash solution (~2/3 of the total capillary volume).

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While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only by the appended claims and equivalents thereof.

CLAIMS

What is claimed is:

1. An automated liquid handling infusion system comprising

a microscale sample holder, said holder configured for holding multiple samples, said holder being situated in conjunction with a positioning system capable of automated operation;

a sample infusion capillary, said capillary having an inlet end and an outlet end, said inlet end of said capillary being in alignment with one of said multiple samples in said sample holder; and

a source of positive or negative pressure for applying said positive or negative pressure across said sample infusion capillary.

2. The liquid handling infusion system of claim 1, wherein said microscale sample holder is a microtiter well plate.

3. The liquid handling infusion system of claim 1, wherein said positioning system is a computer controlled translation stage.

4. The liquid handling infusion system of claim 1, wherein said sample infusion capillary is 2-200 mm long with internal diameter of 2-200 micrometers.

5. The liquid handling infusion system of claim 1, wherein said sample infusion capillary is a capillary array for parallel infusion of several samples.

6. The liquid handling infusion system of to claim 1, wherein said infusion capillary is configured for washing between analyses by a washing device.

7. The liquid handling infusion system of claim 6, wherein a washing reservoir of said washing device is attached to the positioning system.

8. The liquid handling infusion system of claim 6, wherein a washing reservoir of said washing device is a part of the sample holder.

9. The liquid handling infusion system of claim 1, further comprising a chamber coupled to the outlet end of said sample infusion capillary.

10. The liquid handling infusion system of claim 9, wherein the pressure in the chamber can be regulated.

11. The liquid handling infusion system of claim 9, wherein the chamber is an electrospray chamber.

12. The liquid handling infusion system of claim 9, wherein the chamber is a chamber for sample deposition on a movable surface.

13. The liquid handling infusion system of claim 1, wherein said sample infusion capillary further comprises a separation capillary.

14. The liquid handling infusion system of claim 1, wherein said sample infusion capillary is a channel in a microfluidic device.

15. The liquid handling infusion system of claim 14, wherein said channel is open.

16. The liquid handling infusion system of claim 14 where the said channel is filled with an adsorbant packing.

17. A liquid handling reservoir system comprising

a microfluidic device, said device comprising multiple microfabricated channels, each said channel having an inlet end;

a detachable reservoir manifold external to said device, said reservoir having at least one opening for fluid flow into and out of said reservoir, said reservoir positioned so that said reservoir opening is proximate to an inlet end of a microfabricated channel in said device for fluid flow between said reservoir and said channel; and

a source of positive or negative pressure for applying said positive or negative pressure across said system.

18. The reservoir system of claim 17, wherein the volume of the reservoirs is in the range of 10 to 10,000 microliters.

19. The reservoir system of claim 17, wherein the detachable reservoir manifold provides both pneumatic and electrokinetic sample introduction.

20. The reservoir system of claim 17, wherein the detachable reservoir manifold allows washing of the channels on the microdevice.

21. The reservoir system of claim 17, wherein the reservoirs of the manifold are separated from the microfluidic device by a semipermeable membrane.

22. The reservoir system of claim 17, wherein the membrane is used for electroosmotic pumping of the liquid to or from the manifold to the microfluidic device.

23. The reservoir system of claim 17, wherein the membrane is used for sample preconcentration.

24. The reservoir system of claim 17, wherein the membrane contains an immobilized reagent for sample modification.

25. The reservoir system of claim 17, wherein the reagent is an enzyme.

26. The reservoir system of claim 17, wherein the reagent is forming a fluorescence label on the analyzed molecules.

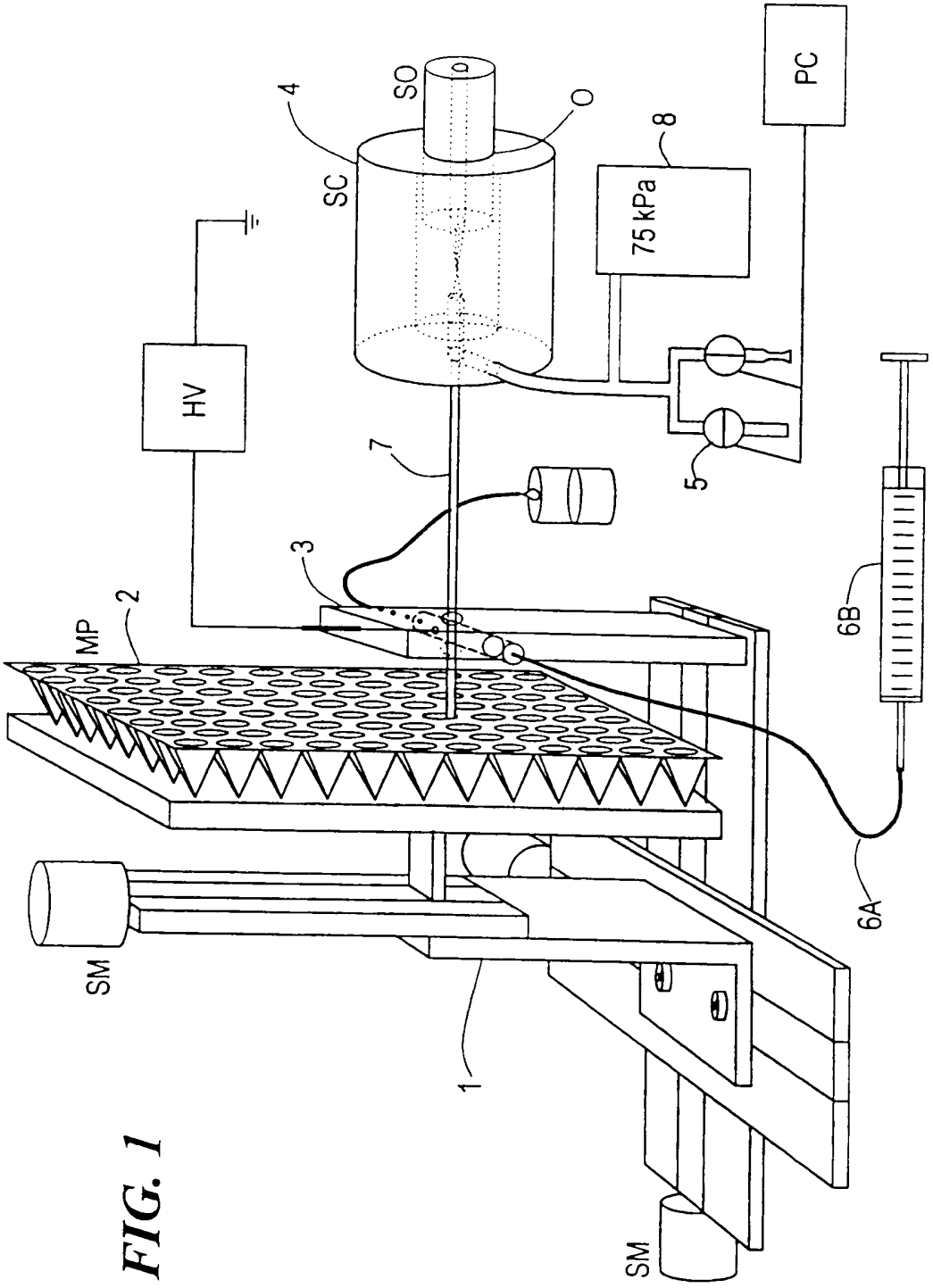

FIG. 1

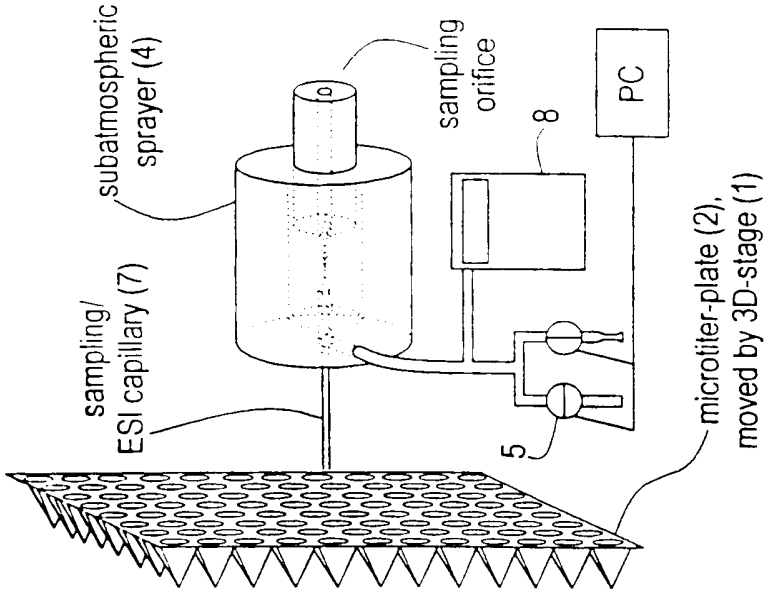

FIG. 2B

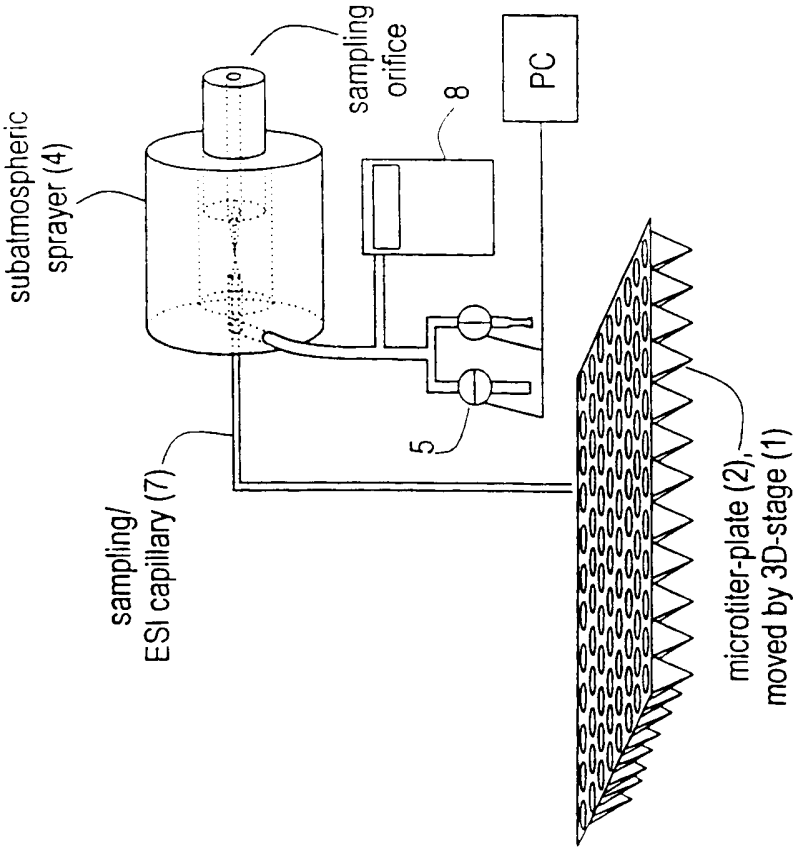

FIG. 2A

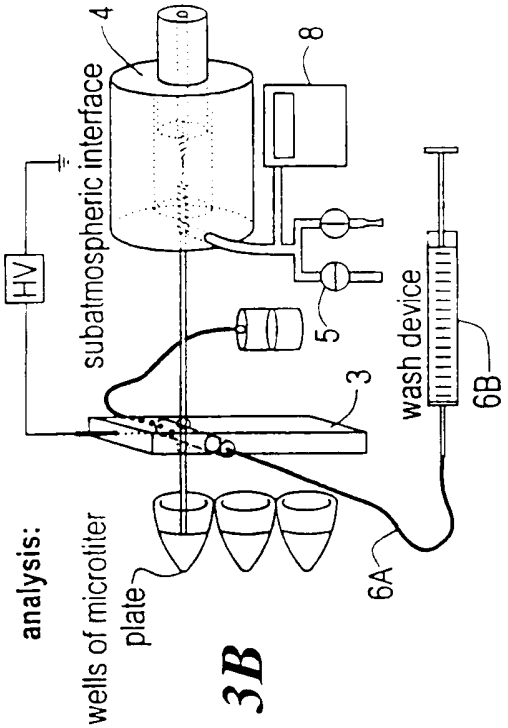

FIG. 3B

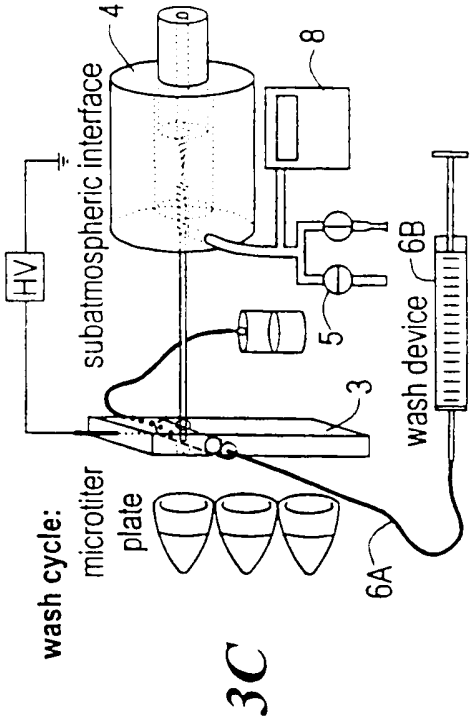

FIG. 3C

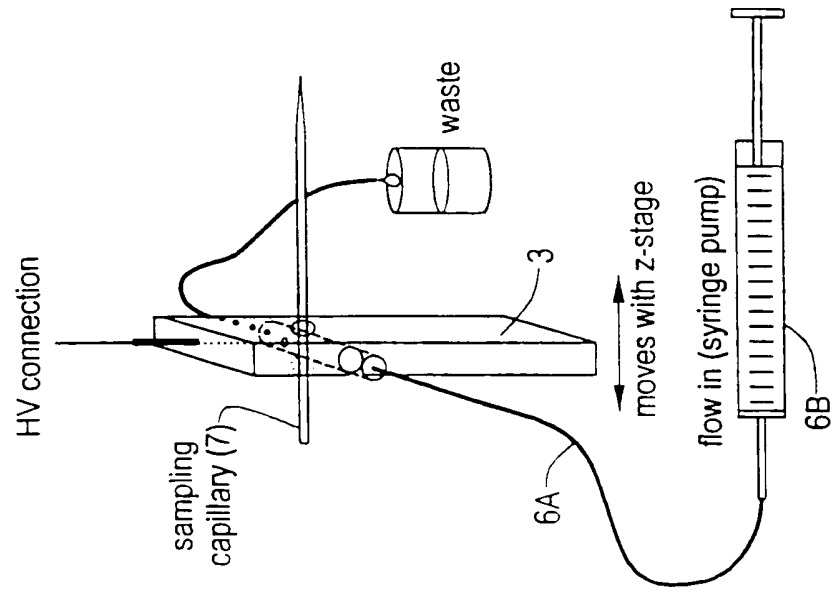

FIG. 3A

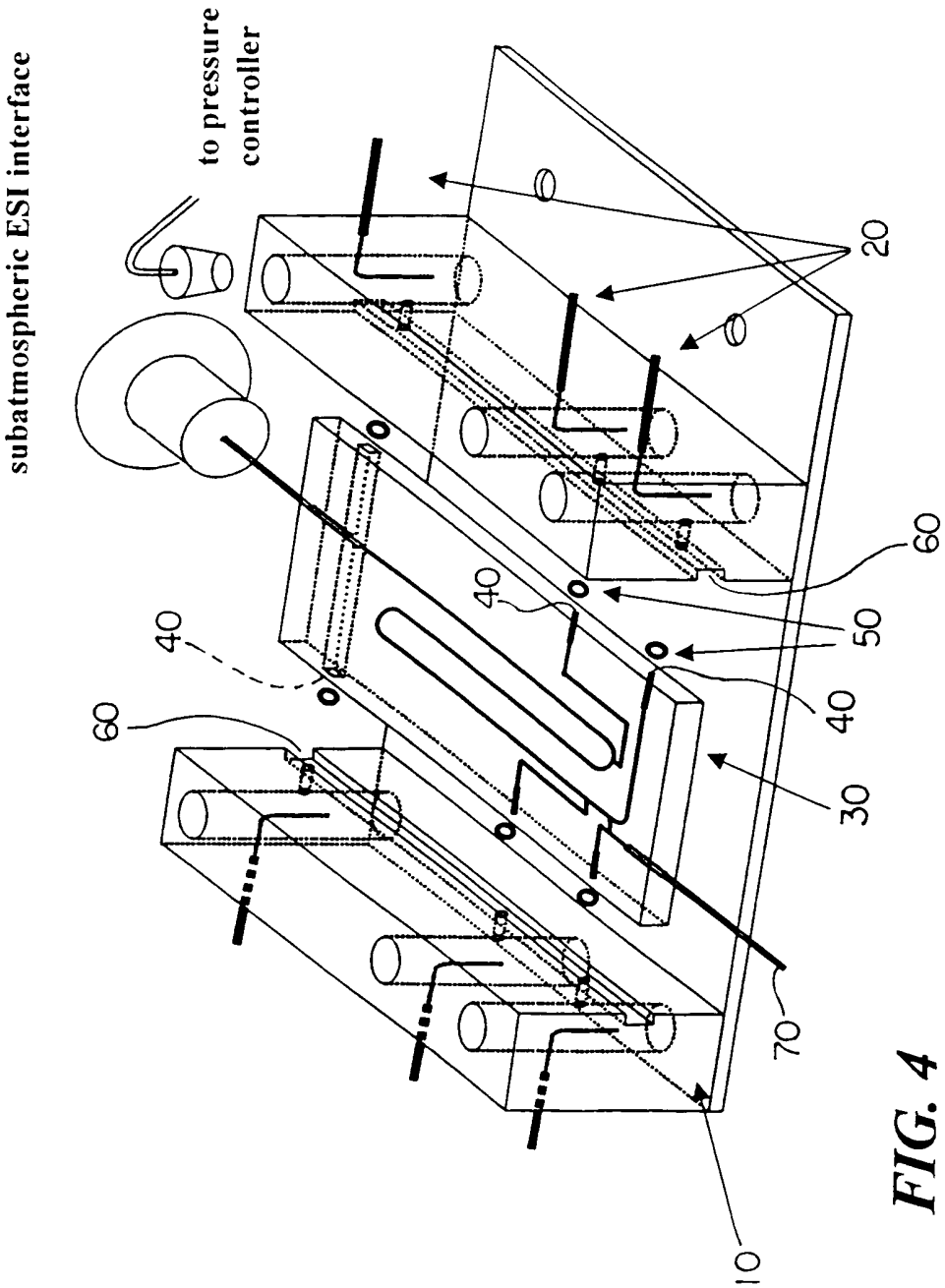

FIG. 4

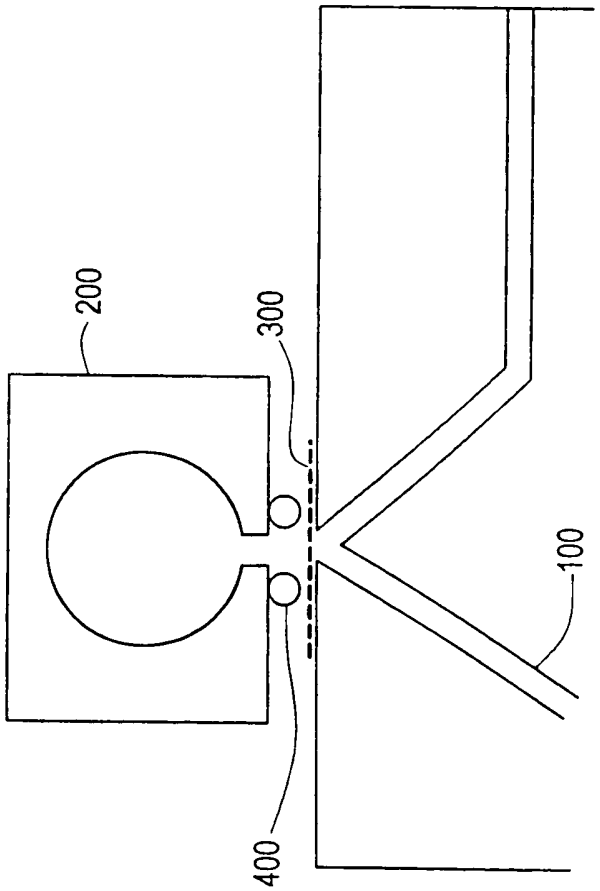

FIG. 5

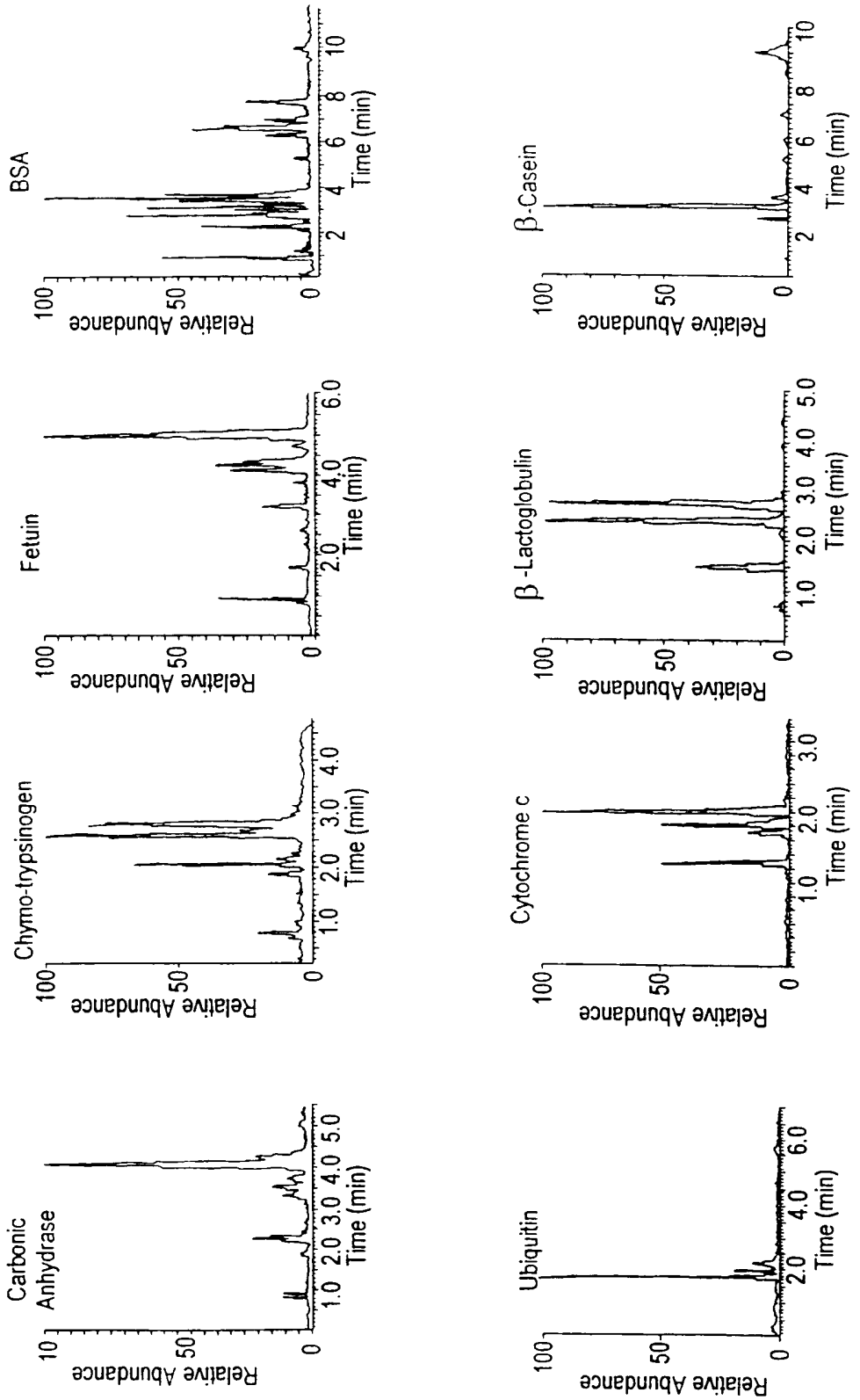

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/09480

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 21/00, 31/00, 33/00; B01L 3/00, 3/02

US CL :422/63, 81, 99, 100

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/63-67, 68.1, 81, 99-104; 436/180; 73/863.01, 863.31, 864.01, 864.11, 864.12, 864.15

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST keyword search on terms: MALDI, liquid dispensing, capillary, microplate, microfluidic, and microchannel

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	US 6,024,925 A (LITTLE et al) 15 February 2000, Figures 1 and 4, columns 6-8.	1,2,4,5,9,10, 12
Y	US 5,872,010 A (KARGER et al) 16 February 1999, Figures 6A and 6B, column 6, lines 10-67, col. 7, l. 1-65.	1 , 3 , 4 , 9 - 12,14,15,17,18
Y	WO 99/14368 A (ADOURIAN et al) 25 March 1999, Figures 1 and 4, page 15, lines 14-38, pages 16-20 all.	1,2,4-6,9,14-16
Y	US 5,498,545 A (VESTAL) 12 March 1996, Figure 8, columns 4-6.	1,2,9,10,12
A	US 5,580,434 A (ROBOTTI et al), 03 December 1996, entire document.	

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

26 JUNE 2000

Date of mailing of the international search report

19 JUL 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

DWAYNE K. HANDY

Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/09480

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,969,350 A (KERLEY et al) 19 October 1999, entire document.	

Form PCT/ISA/210 (continuation of second sheet) (July 1998)*